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(54) Title: OLIGORIBONUCLOSIDE AND OLIGODEOXYRIBONUCLEOSIDE BORANOPHOSPHATES



(57) Abstract

Disclosed are oligonucleoside boranophosphates, or salts thereof, comprising a chain of natural or modified ribonucleotides or deoxyribonucleotides, containing at least one boronated internucleotide phosphodiester linkage of formula (III), wherein W is selected from the group consisting of =O, =S, -OPr, and -SPr where Pr is a base-labile protecting group. X is selected from the group consisting of -BH<sub>3</sub>, -BH<sub>2</sub>R<sub>1</sub>, -BHR<sub>1</sub>R<sub>2</sub> and -BR<sub>1</sub>R<sub>2</sub>R<sub>3</sub>. R<sub>1</sub> is selected from the group consisting of -R<sub>4</sub>, -COOH, -COOR<sub>4</sub>, -CONHR<sub>4</sub>, -CON(R<sub>4</sub>)<sub>2</sub>, -CN+R<sub>4</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>4</sub> is C1 to C18 alkyl. R<sub>2</sub> is selected from the group consisting of -R<sub>5</sub>, -COOH, COOR<sub>5</sub>, -CONHR<sub>5</sub>, -CON(R<sub>5</sub>)<sub>2</sub>, -CN+R<sub>5</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>5</sub> is C1 to C18 alkyl. R<sub>3</sub> is selected from the group consisting of C1 to C3 alkyl. Most preferably, X is -BH<sub>3</sub> and W is =O. Compounds of formula (III) are useful as antisense agents and probes in molecular biology, and have pharmacological activities including anti-inflammatory, antihyperlipidemic, and antineoplastic activity.

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-1-

OLIGORIBONUCLEOSIDE AND  
OLIGODEOXYRIBONUCLEOSIDE BORANOPHOSPHATES

Field of the Invention

The present invention relates to oligonucleotide derivatives having a boron-containing group substituted on an internucleotide phosphodiester linkage.

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Background of the Invention

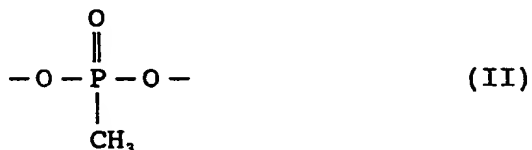
Synthetic oligonucleotides are currently attracting considerable attention as probes for molecular biology and as potential therapeutic agents. See C. Marcus-Sekura, 172 Anal. Biochem. 289 (1988); F. Eckstein, 22 Angew. Chem., Int. Ed. 423 (1983); F. Eckstein, 54 Ann Rev. Biochem. 367 (1985); M. Hamblin et al., 241 Biochem. J. 827 (1987); R. Letsinger et al., 110 J. Am. Chem. Soc. 4470 (1988); P. Miller et al., in Nucleic Acids; The Vectors of Life, 521 (B. Pullman and J. Jortner Eds., 1983); P. Miller and P. Ts'o, 2 Anticancer Drug Design 117 (1987); P. Sarin et al., 85 Proc. Natl. Acad. Sci. USA 7488 (1988); M. Matsukura et al., 86 Proc. Natl. Acad. Sci. USA 4244 (1989); C. Smith et al., 83 Proc. Natl. Acad. Sci. USA 2787 (1985); S. Agrawal et al., 85 Proc. Natl. Sci. USA 7079 (1988). Thus, oligonucleotides with modified backbones, also known as "antisense" agents, may be used to inhibit or control growth of viruses as well as to

specifically control the expression of genes concerned with genetic disorders.

The naturally occurring oligonucleotides and oligodeoxynucleotides contain an internucleotide  
5 phosphodiester linkage of the formula:



Several modifications of the internucleotide phosphodiester linkage have been made. Exemplary is the methylphosphonate, which has the formula:



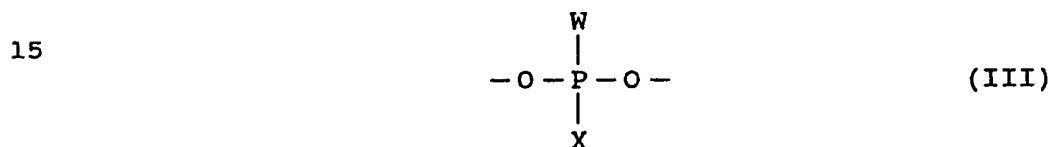
See P. Miller et al., 18 Biochemistry 5134 (1979). Other  
20 modifications which have been made include the phosphotriester (-P-OR), See P. Miller et al., 93 J. Am. Chem. Soc. 6657 (1971); the phosphorothioate (-P-S), See P. Burgers and F. Eckstein, 18 Biochemistry 592 (1979), the phosphorodithioate (S-P-S), See W. Brill et al. 111 J. Am. Chem. Soc. 2321 (1989), and the phosphoramidate (-P-NR<sub>2</sub>),  
25 See K. Ogilvie and J. Nemer, 21 Tetrahedron Lett. 4145 (1988). Such modified oligonucleotides have been shown to inhibit viruses such as HIV, HSV, etc., and the expression of oncogenes such as c-myc and c-Ha-ras. See C. Stein and  
30 J. Cohen, 48 Cancer Res. 2659 (1988); C. Markus-Sekura et al. 15 Nucl. Acids Res. 5749 (1987); R. Meikkila et al., 328 Nature 445 (1987).

While it is clear that considerable potential exists for modified oligonucleotides as both probes and  
35 potential therapeutic agents, it is equally clear that a considerable effort is required to translate this field

into new and useful compounds. The present invention arose from our ongoing research into new oligonucleotides useful as gene probes and as potential therapeutic agents.

### Summary of the Invention

5 We herein disclose the first examples of oligonucleotides with a boronated internucleotide phosphodiester linkage, thus establishing an entirely new class of nucleic acid derivatives. More particularly, disclosed herein is an oligoribonucleoside or oligodeoxy-  
10 ribonucleoside boranophosphate, or a salt thereof, comprising a chain of natural or modified nucleotides or deoxynucleotides containing at least one boronated internucleotide phosphodiester linkage of the formula:



wherein:

20 W is selected from the group consisting of =O, =S, -OPr, and -SPr where Pr is a base-labile protecting group (i.e., leaving group) which can be removed from the oxygen or sulfur by a strong base to yield =O or =S as W during the synthesis of the oligonucleotide by the  
25 phosphite-triester method. Preferably, W is selected from the group consisting of =O, =S, -OR', -SR', and -OCH<sub>2</sub>CH<sub>2</sub>CN, wherein R' is C1 to C3 alkyl, preferably methyl. Compounds in this group having -OR', -SR', or -OCH<sub>2</sub>CH<sub>2</sub>CN as W are useful as intermediates for making compounds having as W =O  
30 or =S, and compounds in this group having =O, =S, -OR' and -SR' are useful as products in the methods of use discussed below. Compounds having =O as W are the most preferred products, and compounds having -OCH<sub>3</sub> as W are the most preferred intermediates.

35 X is selected from the group consisting of -BH<sub>3</sub>, -BH<sub>2</sub>R<sub>1</sub>, -BHR<sub>1</sub>R<sub>2</sub> and -BR<sub>1</sub>R<sub>2</sub>R<sub>3</sub>. Preferably, X is -BH<sub>3</sub>,

-BH<sub>2</sub>R<sub>1</sub>, or -BHR<sub>1</sub>R<sub>2</sub>. More preferably, X is -BH<sub>3</sub> or -BH<sub>2</sub>R<sub>1</sub>.  
Most preferably, X is -BH<sub>3</sub>.

R<sub>1</sub> is selected from the group consisting of  
-R<sub>4</sub>, -COOH, -COOR<sub>4</sub>, -CONHR<sub>4</sub>, -CON(R<sub>4</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>4</sub>, -CN,  
5 carboxycholesteryl and carboxybenzyl, wherein R<sub>4</sub> is C1 to  
C18 alkyl. Preferably R<sub>1</sub> is -R<sub>4</sub>, -COOH, -COOR<sub>4</sub>, -CONHR<sub>4</sub>,  
-CON(R<sub>4</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>4</sub>, or -CN; more preferably R<sub>1</sub> is -R<sub>4</sub>, -COOH,  
-COOR<sub>4</sub>, -CONHR<sub>4</sub>, -CON(R<sub>4</sub>)<sub>2</sub>, or -CN; most preferably R<sub>1</sub> is  
-R<sub>4</sub> or -CN. Preferably R<sub>4</sub> is C1 to C9 alkyl and more  
10 preferably R<sub>4</sub> is C1 to C3 alkyl.

R<sub>2</sub> is selected from the group consisting of  
-R<sub>5</sub>, -COOH, -COOR<sub>5</sub>, -CONHR<sub>5</sub>, -CON(R<sub>5</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>5</sub>, -CN,  
carboxycholesteryl and carboxybenzyl, wherein R<sub>5</sub> is C1 to  
C18 alkyl. Preferably R<sub>2</sub> is -R<sub>5</sub>, -COOH, -COOR<sub>5</sub>, -CONHR<sub>5</sub>,  
15 -CON(R<sub>5</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>5</sub>, or -CN; more preferably R<sub>2</sub> is -R<sub>5</sub>, -COOH,  
-COOR<sub>5</sub>, -CONHR<sub>5</sub>, -CON(R<sub>5</sub>)<sub>2</sub>, or -CN; still more preferably R<sub>2</sub>  
is -R<sub>5</sub> or -COOH, and most preferably R<sub>2</sub> is -R<sub>5</sub>. Preferably  
R<sub>5</sub> is C1 to C9 alkyl; more preferably R<sub>5</sub> is C1 to C3 alkyl;  
and most preferably R<sub>5</sub> is methyl.

20 R<sub>3</sub> is selected from the group consisting of C1 to  
C3 alkyl, preferably methyl.

Oligonucleotides and oligodeoxynucleotides  
containing a boronated internucleotide phosphodiester  
linkage represent an important, and perhaps ideal,  
25 "antisense" species in that the nucleotide bases are  
unaltered (thus maintaining binding specificity) and the  
backbone may remain negatively charged (for water  
solubility) like the natural O-oligos of Formula (I).  
Since BH<sub>3</sub> is much more hydrophobic than oxygen, it should  
30 impart a greater membrane permeability than the O-oligo and  
yet maintain nuclease resistance like the  
methylphosphonates illustrated in formula (II). Although  
compounds containing boron-hydride bonds are susceptible to  
hydrolysis, the B-H bond in boranophosphates possesses  
35 unusual hydrolytic stability. Moreover, since these  
nucleic acids contain boron, the additional advantage of  
using the unique neutron capturing ability of the non-

radioactive  $^{10}\text{B}$  isotope, R. G. Fairchild and G. L. Brownell, Editors "Proceedings of the First International Symposium on Neutron Capture Therapy", October 12-14, 1983, as a label in a probe or as a therapeutic agent also exists.

- 5 For this purpose, boron used to make the compounds of the present invention may be  $^{10}\text{B}$  enriched.

#### Detailed Description of the Invention

- Oligonucleotides of the present invention may, apart from the inventive boron-containing linkage, be  
10 polymers of the naturally occurring nucleotides, modified nucleotides, or of both naturally occurring and modified nucleotides. Modifications may be to the internucleotide phosphodiester linkages, to the nucleosides, or to both.

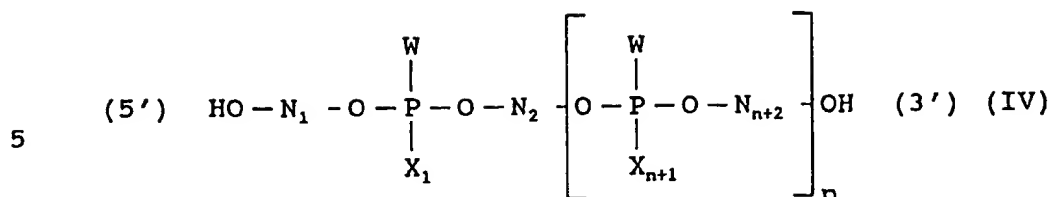
- Thus, the nucleoside base may be a natural base,  
15 such as adenine, thymine, cytosine, guanine, uracil, xanthine, or hypoxanthine, (the latter two being the natural degradation products) or in derivatives may be the base of a modified nucleic acid such as 4-acetylcytidine; 5-(carboxyhydroxymethyl)uridine; 2'-O-methylcytidine;  
20 5-carboxymethylaminomethyl-2-thiouridine; 5-carboxymethylaminomethyluridine; dihydrouridine; 2'-O-methylpseudo-uridine; beta,D-galactosylqueosine; 2'-O-methylguanosine; N6-isopentenyladenosine; 1-methyladenosine; 1-methylpseudo-uridine; 1-methylguanosine; 1-methylinosine; 2,2-dimethyl-  
25 guanosine; 2-methyladenosine; 2-methylguanosine; 3-methylcytidine; 5-methylcytidine; N6-methyladenosine; 7-methylguanosine; 5-methylaminomethyluridine; 5-methoxyaminomethyl-2-thiouridine; beta,D-mannosylqueosine; 5-methoxycarbonylmethyluridine; 5-methoxyuridine; 2-methylthio-N6-  
30 isopentenyladenosine; N-((9-beta-D-ribofuranosyl-2-methylthiopurine-6-yl)carbamoyl)threonine; N-((9-beta-D-ribofuranosylpurine-6-yl)N-methyl-carbamoyl)threonine; uridine-5-oxyacetic acid methylester; uridine-5-oxyacetic acid (v); pseudouridine; queosine; 2-thiocytidine; 5-  
35 methyl-2-thiouridine; 2-thiouridine; 4-thiouridine; 5-methyluridine; 2'-O-methyl-5-methyluridine; and 2'-O-methyluridine.

Derivatives may be formed by modifying the internucleotide phosphodiester linkage. Internucleotide phosphodiester linkages in the chain may be modified, for example, to the methylphosphonate, the phosphotriester, the phosphorothioate, the phosphorodithioate, and the phosphoramidate (see Formula II above and accompanying text).

Derivatives may be formed by modifying the 3' or 5' end of the oligonucleotide. Groups which can be added to the 3' or 5' end vary widely, from relatively inert protecting groups to reactive groups or groups with special properties to obtain desirable physical, chemical, or biochemical effects. For example, derivatives may be formed by joining an intercalating agent to the oligonucleotide in the manner described in U. S. Patent No. 4,835,263 to Nguyen et al. (the disclosure of this and all other patent references cited herein is to be incorporated herein by reference). A wide variety of protecting groups can be substituted on the 5' and 3' hydroxy groups (e.g., of compounds of Formula (IV) below), such as the triphenylmethyl (trityl) group and its derivatives on the 5' hydroxy group, and acetyl, benzoyl, or the 3'-O-succinyl group on the 3' hydroxy group, as known in the art. See, e.g., 1 Chemistry of Nucleosides and Nucleotides, 287-92 (L. Townsend ed. 1988). In general, the 5' hydroxy group is protected with an acid labile group and the 3' hydroxy group is protected with an acyl group. Id. at 289 (When the 5' hydroxyl group is protected with an acid labile group such as mono- and dimethoxytrityl, the 3'-hydroxyl group of deoxynucleosides can be protected with acyl groups.).

A preferred group of oligonucleoside boranophosphates, or salts thereof, of Formula (III) above have the formula, from 5' to 3', of Formula (IV):





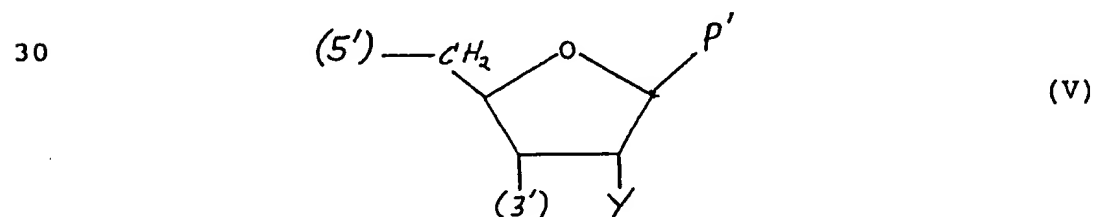
In Formula (IV) The value of  $n$  is not critical. Typically,  $n$  is an integer having a value of from 0 to 50, depending upon the number and placement of boron-containing groups and the particular synthetic scheme employed. When  $n$  is 0 the bracketed group is absent and  $\text{N}_2$  is bound to the 3' -OH group, as shown. Preferably,  $n$  has a value of from 0 to 30, more preferably  $n$  has a value of from 0 to 18, and most preferably,  $n$  has a value of from 0 to 3.

$W$  in Formula (IV) is as given in connection with formula (III) above.

$\text{X}_1$  through  $\text{X}_{n+1}$  in Formula (IV) are each independently selected from the group consisting of -OH, -BH<sub>3</sub>, -BH<sub>2</sub>R<sub>1</sub>, -BHR<sub>1</sub>R<sub>2</sub> and -BR<sub>1</sub>R<sub>2</sub>R<sub>3</sub>, subject to the proviso that at least one of  $\text{X}_1$  through  $\text{X}_{n+1}$  is not -OH. The -OH group may, of course, exist as -O<sup>-</sup> in salts of the compounds of formula (IV). Preferably, at least  $\text{X}_1$  and  $\text{X}_{n+1}$  are not -OH. Most preferably,  $\text{X}_1$  through  $\text{X}_{n+1}$  are not -OH.

$\text{R}_1$ ,  $\text{R}_2$ , and  $\text{R}_3$  in Formula (IV) are as given in connection with formula (III) above.

$\text{N}_1$  through  $\text{N}_{n+2}$  are each independently a nucleoside or deoxynucleoside monomer of the formula:



$Y$  is selected from the group consisting of -H (the deoxyribonucleoside) and -OH (the ribonucleoside). Preferably  $Y$  is -H.

P' is a base of a natural or modified nucleic acid, which are discussed in detail above above.

Preferably, P' is selected from the group consisting of adenine, thymine, cytosine, guanine, uracil, xanthine, hypoxanthine, 5-methylcytosine, 5-bromouracil, 8-azidoadenine, and 2,6-diaminopurine. Most preferably, P' is adenine, thymine, cytosine, guanine, or uracil.

In nucleosides and deoxynucleosides, the pentose is joined to the base by a  $\beta$ -N-glycosyl bond between carbon atom 1 of the pentose and either nitrogen atom 9 of purine bases (and their derivatives) or nitrogen atom 1 of pyrimidine bases (and their derivatives). The phosphate group of a nucleotide is in ester linkage with carbon atom 5 of the pentose. In a polynucleotide chain, the phosphate group of a nucleotide is additionally in ester linkage with carbon atom 3 of the pentose in the adjacent nucleotide to form the phosphodiester internucleotide bond.

The term "alkyl," as used herein, refers to linear or branched alkyl groups which may be saturated or unsaturated. Preferred alkyl groups in the compounds of Formula (III) are linear and saturated.

The following compounds are exemplary of the compounds of Formula (III):

(A) thymidylyl[3'-5']-(3'-acetylthymidylyl) (borane)phosphonate methyl ester (or 3'-O[thymidylyl]-5'-O-[3'-acetylthymidylyl]methyl phosphite-borane);

(B) thymidylyl[3'-5']-(3'-acetylthymidylyl) (cyanoborane)phosphonate methyl ester;

(C) thymidylyl[3'-5']-(3'-acetylthymidylyl) (carbomethoxyborane)phosphonate methyl ester;

(D) thymidylyl[3'-5']-(3'-acetylthymidylyl) (N-ethylcarbamoylborane)phosphonate methyl ester;

(E) thymidylyl[3'-5']-(3'-acetylthymidylyl) (carboxyborane)phosphonate methyl ester;

(F) thymidylyl[3'-5']-(3'-acetylthymidylyl) (N-ethylcarbamoyl)methylborane phosphonate methyl ester;

(G) 2'deoxyadenylyl[3'-5']-(3'acetyl-2'deoxyadenylyl)boranephosphonate methyl ester;

(H) 2'deoxyguanylyl[3'-5']-(3'acetyl-2'deoxyguanylyl)boranephosphonate methyl ester;

5 (I) 2'deoxyguanylyl[3'-5']-(3'-acetyl-2'-deoxyguanylyl)(cyanoborane) phosphonate methyl ester;

(J) thymidylyl[3'-5']-thymidylyl(borane)-phosphonate, ammonium salt;

10 (K) thymidylyl[3'-5']-thymidylyl(cyanoborane)-phosphonate, ammonium salt;

(L) thymidylyl[3'-5']-thymidylyl(carbomethoxy)-phosphonate, ammonium salt;

(M) thymidylyl[3'-5']-thymidylyl(methylborane)-phosphonate, ammonium salt;

15 (N) thymidylyl[3'-5']-thymidylyl(N-ethyl-carbamoyl)methylborane phosphonate, ammonium salt;

(O) 2'deoxycytidylyl[3'-5']-2'deoxycytidylyl-(borane)phosphonate ammonium salt;

20 (P) 2'deoxycytidylyl[3'-5']-2'deoxycytidylyl-(cyanoborane)phosphonate ammonium salt;

(Q) 2'deoxyadenylyl[3'-5']-2'deoxyadenylyl-(cyanoborane)phosphonate ammonium salt;

(R) thymidylyl[3'-5']2'-deoxyadenylyl[3'-5']-2'-deoxyguanylyl(borane)phosphonate dimethyl ester;

25 (S) thymidylyl[3'-5']2'-deoxyadenylyl[3'-5']-2'-deoxyguanylyl(cyanoborane)phosphonate dimethyl ester;

(T) thymidylyl[3'-5']thymidylyl[3'-5'](3'-acetylthymidylyl) boranephosphonate dimethyl ester;

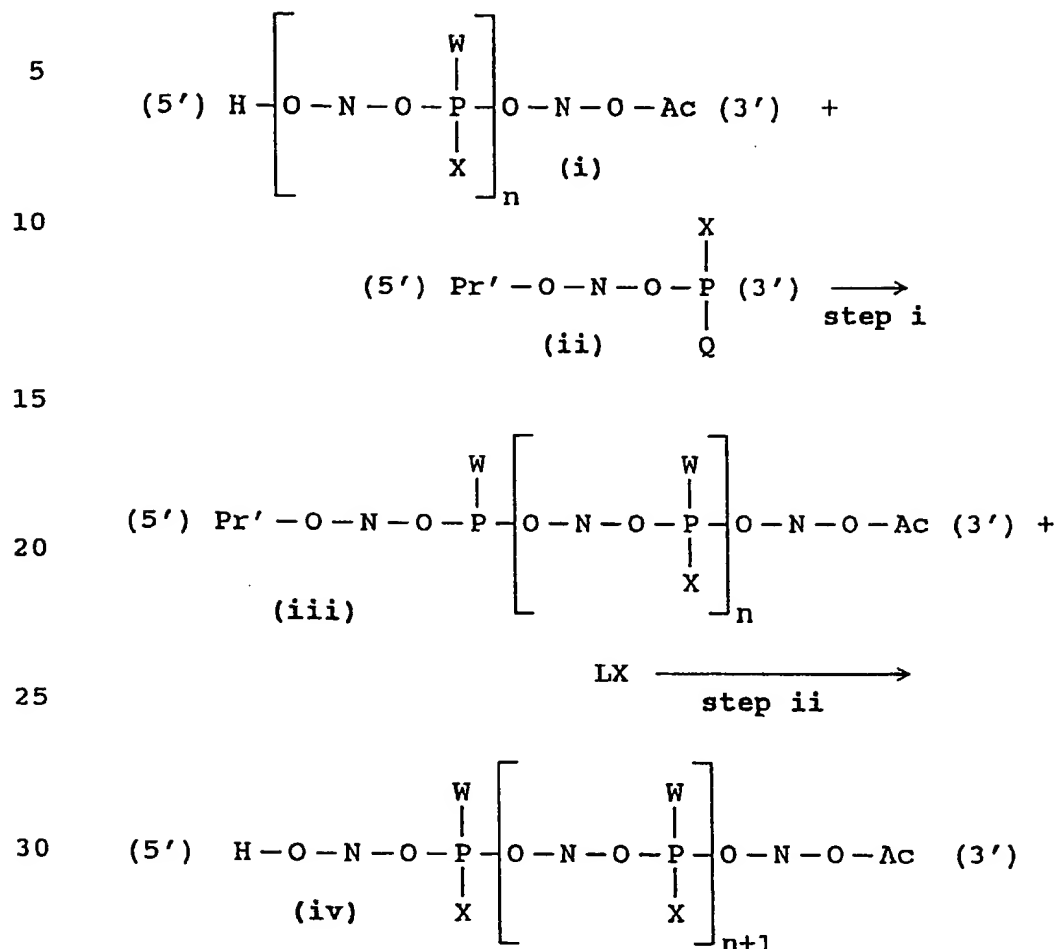
30 (U) thymidylyl[3'-5']thymidylyl-boranephosphonate[3'-5'](3'-acetylthymidylyl) cyanoboranephosphonate dimethyl ester;

(V) tetra(thymidine boranephosphonate); and

(W) hexa(adenine boranephosphonate).

35 Compounds of the present invention are preferably made by the reactions shown in Scheme 1 below.

## Scheme 1



As shown in Scheme 1, boronated oligonucleotides  
 of the present invention are prepared by a modification of  
 the phosphite-triester method, see generally 1 Chemistry of  
 Nucleosides and Nucleotides, 310-19 (L. Townsend Ed. 1988),  
 in which the step of oxidizing the phosphite linkage of the  
 intermediate (step ii) to form a phosphate linkage is  
 carried out with an adduct of a Lewis base and a boron-  
 containing group (LX). Ordinarily, the phosphite-triester  
 method involves a chain elongation condensation reaction,  
 an oxidation reaction to convert the intermediate phosphite  
 to the phosphate, and a 5' deprotection step so that the

chain elongation reaction may be repeated and the cycle of steps continued. When the reaction described herein is carried out with excess adduct, the 5' deprotection step may be advantageously eliminated.

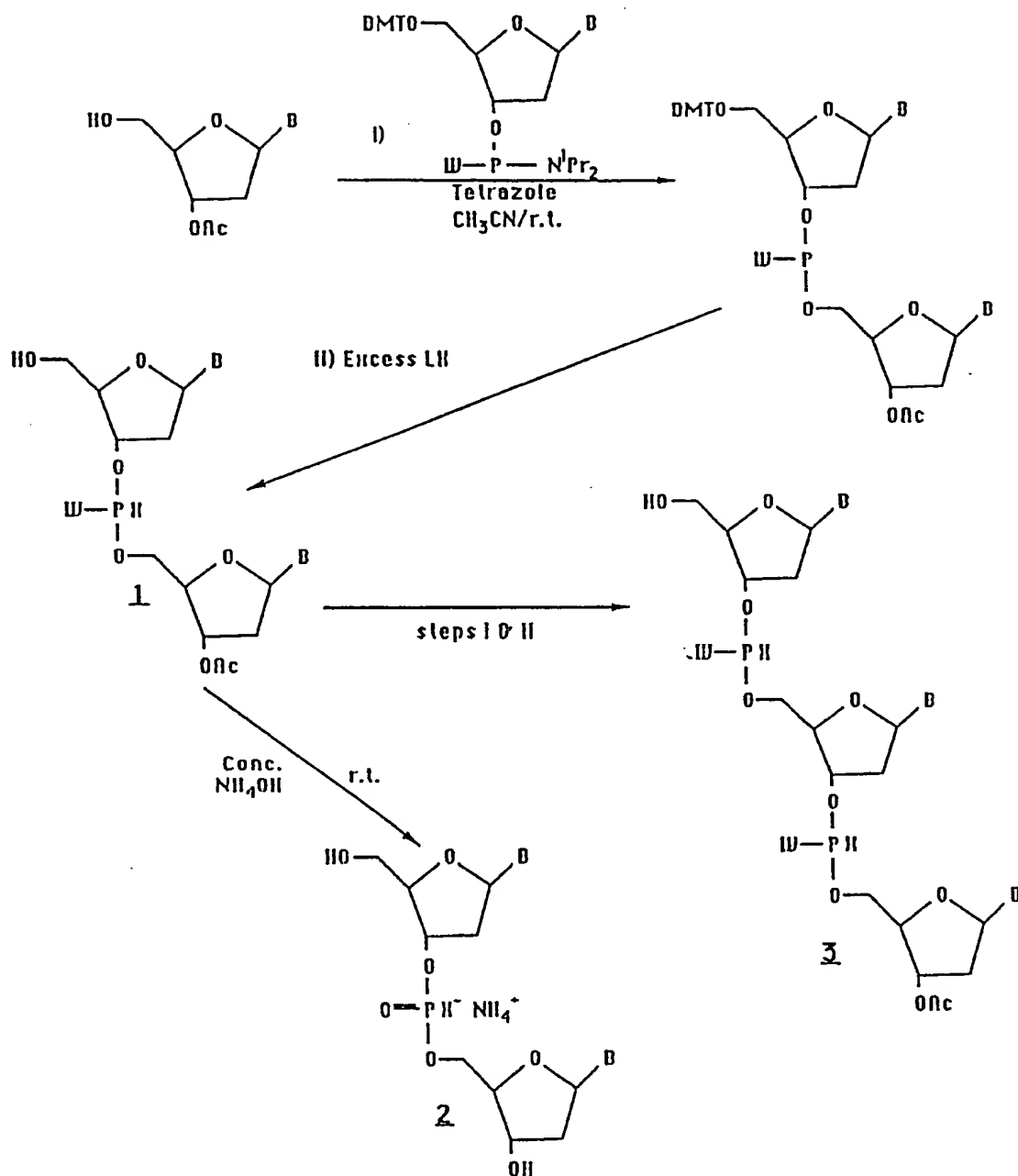
5           As shown in scheme 1, compound (i) is condensed with compound (ii) in step i to give compound (iii); compound (iii) is oxidized with LX in step ii to give compound (iv). To continue the chain elongation cycle, step i is repeated with compound (iv) taking the place of  
10 compound (i), and step ii then optionally repeated. This cycle of steps is repeated until a chain of the desired length, as discussed in connection with Formula (iv) above, is reached. In the first cycle, n is zero.

          Pr' represents any 5' protecting group, as  
15 discussed above, but is preferably Lewis acid labile. Ac represents an acyl group (for liquid phase synthesis) or a solid support (for solid phase synthesis), as also discussed above. X represents Pr-V, where Pr is a protecting group and V is S or O (C being covalently bound to P), as given in connection with Formula III above. Q is  
20 a proton accepting group which is a stronger proton acceptor than the 5' oxygen in compound (i). Any protecting group and proton accepting group may be employed which is employed in the phosphite-triester method, with  
25 Pr and Q determined by the particular phosphitylating reagent employed. Phosphitylating reagents have the general formula L-V-Q. See Chemistry of Nucleosides and Nucleotides, 310-14 (L. Townsend Ed. 1988). The phosphitylating reagent is not shown in Scheme 2, but may  
30 be any phosphitylating reagent which may be reacted with a 5'-protected nucleoside to prepare compound (ii). Thus, Pr may for example be C1 to C3 alkyl, 2,2,2-tribromoethyl-, benzyl-, 2-cyanoethyl-, 2-methylsulfonyl-, 4-nitrophenylethyl, 2-chlorophenyl, p-chlorophenyl-,  
35 phenylethyl- or p-nitrophenylethyl-, and Q may for example be chlorine, 1-substituted 1,2,4-triazole, or 1-substituted 1,2,3,4-tetrazole. See Id.

-12-

A particularly preferred method of preparing compounds of the present invention is given in Scheme 2 below.

Scheme 2



-13-

In scheme 2, the substituents W and X are as given above. DMT refers to a dimethoxytrityl protecting group, but may be any suitable 5' protecting group as discussed above. Ac is an acyl group such as acetyl (Ac represents a solid support in solid-phase synthesis, as discussed in greater detail below). The substituent B on the ribose refers to the base purine, pyrimidine, or derivative thereof, as identified by substituent P' in connection with Formula (V) above.

10 In step (i) of Scheme 2, a 5'-DMT-nucleoside phosphoramidite is reacted with a 3'-acylnucleoside in the presence of tetrazole to form an intermediate phosphite, which phosphite is converted in step (ii) to a 3'-  
15 acyloligonucleotide (1) having an internucleotide phosphodiester linkage as given in Formula (III) above by oxidation with LX, where L is a Lewis base, such as  $-S(CH_3)_2$  or  $-N(CH_3)_3$ , which is a weaker electron donors than the internucleotide phosphorus (The strong Lewis base properties of Trialkylphosphites are described in T. Reetz,  
20 Trialkyl Phosphite Borines. A New Type of Phosphorus-boron Compound, J. Am. Chem. Soc. **82**, 5039 (1960)). For chain elongation, steps (i) and (ii) are then repeated, with the product of step (ii) being reacted with a 5'-DMT-nucleoside, to form oligonucleotides of greater length  
25 (e.g., the trimer 3). Alternatively, the 3'-acyloligonucleotide (1) may be hydrolyzed with a strong base to remove the 3' acyl group and restore the 3' hydroxyl group as in compound (2), and remove the protecting group Pr in X.

30 Solid phase synthesis for the above-described scheme is implemented by esterifying the 3' carbon of the substrate nucleotide to an insoluble support instead of an acyl group (as is done for liquid phase synthesis). Solid phase synthesis for the phosphite-triester method is known,  
35 and the insoluble support may be any of those conventionally employed, such as controlled pore glass.

See generally 1 Chemistry of Nucleosides and Nucleotides, 314-19 (L. Townsend ed. 1988).

Side reactions with bases, e.g., when the base is Adenine or Cytosine (hydroboration of the carbon-carbon double bond) may be eliminated by replacing  $\text{Me}_2\text{SX}$  in the substitution step with  $\text{Me}_3\text{NX}$  since  $\text{Me}_3\text{NX}$  is a much weaker hydroboration agent than  $\text{Me}_2\text{SX}$ . While phosphites easily displace trimethylamine from  $\text{Me}_3\text{NX}$ , weakly basic nucleoside bases will not react with this compound. Other borane sources, such as  $\text{Ph}_3\text{PX}$  or aniline borane may also give satisfactory results.

When the boronating agent is a substituted borane, such as  $\text{Me}_2\text{SBH}_2\text{CN}$ , since the  $\text{BH}_2\text{CN}$  species is a less powerful hydroborating agent, hydroborated side products with the C-C double bond with Adenine or Cytosine should be greatly lessened.

Since the phosphorus donor base in  $(\text{RO})_3\text{P}$  is stronger than amine, ethers, sulfides, etc. (T. Reetz, J. Amer. Chem. Soc. 82, 5039 (1960)) even if initial formation of a purine or pyrimidine nitrogen base·X adduct takes place, the thermodynamically more stable phosphorus·X will eventually be formed. Additionally, since the P·X species are expected to possess high hydrolytic stability, any purine or pyrimidine nitrogen base·X adduct may be removed by appropriate hydrolysis with acid or base.

The reaction of an intermediate dinucleotide phosphite with  $\text{Ph}_3\text{PBH}_2\text{CN}$  or  $\text{PhNH}_2\text{BH}_2\text{CN}$  has been carried out at room temperature and 68°C without success. It appears that the  $\text{Ph}_3\text{P}$  or  $\text{NH}_2\text{Ph}$  are more difficult to displace than  $\text{Me}_2\text{S}$ . Thus, by using a weaker Lewis base ( $\text{Me}_2\text{S}$ ) adduct of  $\text{BH}_2\text{CN}$ , i.e.,  $\text{Me}_2\text{SBH}_2\text{CN}$  or  $(\text{BH}_2\text{CN})_x$  itself, formation of the phosphorus- $\text{BH}_2\text{CN}$  should readily take place.

Compounds of Formula (III) which are capable of binding to polyribonucleic acid or polydeoxyribonucleic acid are useful as antisense agents in the same manner as conventional antisense agents. See generally Antisense Molecular Biology and S-oligos, Synthesis 1 (Oct. 1988)



(published by Synthecell Corp., Rockville, MD); 2  
Discoveries in Antisense Nucleic Acids (C. Brakel and R.  
Fraley eds. 1989). Antisense agents of the present  
invention may be used by contacting an antisense agent  
5 which is capable of selectively binding to a predetermined  
polydeoxyribonucleic acid sequence or polyribonucleic acid  
sequence to a cell containing such sequence (e.g., by  
adding the antisense agent to a culture medium containing  
the cell) so that the antisense agent is taken into the  
10 cell, binds to the predetermined sequence, and blocks  
transcription, translation, or replication thereof. The  
requirements for selective binding of the antisense agent  
are known (e.g., a length of 17 bases for selective binding  
within the human genome).

15           Compounds of Formula (III) which are capable of  
binding to polydeoxyribonucleic acid or polyribonucleic  
acid are useful as probes in molecular biology. These  
probes can be used in any suitable environment, such as  
Southern blotting and Northern blotting, the details of  
20 which are known. See, e.g., R. Old and S. Primrose,  
Principles of Gene Manipulation, 8-10 (3d Ed. 1985). When  
used as probes, the boron atom serves essentially as a  
radiolabel, though it is not itself radioactive until  
exposed to thermal neutron radiation (low energy neutrons).  
25 When exposed to low energy neutrons,  $^{10}\text{B}$  absorbs a neutron  
and forms  $^{11}\text{B}$ , which rapidly decays and releases an alpha  
particle, thus providing a detectable signal. The  
techniques involved in the generation of the alpha particle  
are known. See, e.g., A. Soloway, Borax Rev. 3, 7-9  
30 (1988).

Probes of the present invention can be used by  
contacting the probe (which is capable of selectively  
binding to a predetermined polydeoxyribonucleic acid or  
polyribonucleic acid sequence) to a substrate DNA or RNA  
35 sample suspected of containing the predetermined sequence  
so that the compound of Formula (III) will bind to the  
predetermined sequence if the predetermined sequence is

present. The requirements for selective binding are known, as is the case with antisense agents. Next, unbound probe is removed from the substrate, and the substrate is exposed to thermal radiation. The release of alpha particles from the substrate indicates that the predetermined sequence is present in the substrate. The procedure may be carried out on single-stranded substrate DNA which is immobilized on a nitrocellulose support, which support is then placed in a solution containing the probe, then washed, then placed in contact with photographic film and exposed to thermal radiation, as in a Southern blot. The procedure may be carried out in like manner with an RNA substrate covalently bound to reactive paper (e.g., paper prepared by diazotization of aminobenzyloxymethyl-paper), as in a Northern blot. Conventional techniques for neutron capture radiography may be employed. See D. Gabel et al., Cancer Res. 47, 5451 (1987).

Boron probes may be detected in vivo by  $^{11}\text{B}$  multinuclear magnetic resonance imaging (MRI) and spectroscopy, see, e.g., G. Kabalka et al., Magnetic Resonance in Medicine 8, 231 (1988), by administering the probe to an animal subject and detecting the probe by  $^{11}\text{B}$  MRI, in accordance with known procedures.

The compounds of the present invention have pharmaceutical activity, including anti-inflammatory, antihyperlipidemic, and antineoplastic activity.

A method of combatting hyperlipidemia in an animal subject comprises administering an animal subject in need of such treatment a hyperlipidemia-combatting amount of a compound of Formula (III).

A method of producing an anti-inflammatory response in an animal subject in need of such treatment comprises administering an animal subject an inflammation-combatting amount of a compound of Formula (III).

A method of combatting tumors, preferably solid tumors (e.g., adenocarcinoma, bronchogenic carcinoma, osteosarcoma, epidermoid carcinoma, breast carcinoma,

glioma), in an animal subject in need of such treatment comprises administering an animal subject a tumor-combatting amount of a compound of Formula (III), after which the tumor is preferably exposed to thermal radiation (low energy neutrons) in an amount effective for  $^{10}\text{B}$  located in the tumor by virtue of the administration of the compound of Formula (III) to the subject to capture a neutron, decay, and release an alpha particle in cells of the tumor.

Subjects to be treated by the methods of the present invention include both human and animal (e.g., bird, dog, cat, cow, horse) subjects, and are preferably mammalian subjects.

Animal subjects are administered compounds of Formula (III) at a daily dose of preferably at least about .1 mg/kg, more preferably at least about .5 mg/kg, and most preferably at least about 2 mg/kg. The daily dose is preferably not more than about 1000 mg/kg, more preferably not more than about 200 mg/kg, and most preferably not more than about 50 mg/kg.

As noted above, the compounds of Formula (III) may be administered per se or in the form of a pharmaceutically acceptable salt. When used in medicine, the salts of the compound of Formula (III) should be both pharmacologically and pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare the free active compound or pharmaceutically acceptable salts thereof and are not excluded from the scope of this invention. Where appropriate, such pharmacologically and pharmaceutically acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluenesulfonic, tartaric, citric, methanesulphonic, formic, malonic, succinic, naphthalene-2-sulphonic and benzenesulphonic. Also, where appropriate, pharmaceutically acceptable salts can be prepared as

alkaline metal or alkaline earth salts, such as the sodium, potassium or calcium salts of a carboxylic acid group.

The present invention also provides pharmaceutical formulations, both for veterinary and for human medical use, which comprise the active agent (the compound of Formula (III)) together with one or more pharmaceutically acceptable carriers thereof and optionally any other therapeutic ingredients. The carrier(s) must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the formulation and not unduly deleterious to the recipient thereof. The active agent is provided in an amount effective to achieve the desired pharmacological effect, as described above, in a quantity appropriate to achieve the desired daily dose.

The formulations include those suitable for oral, rectal, topical, nasal, ophthalmic or parenteral (including subcutaneous, intramuscular and intravenous) administration. Formulations suitable for parenteral administration are preferred.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active compound into association with a carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product into desired formulations.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, tablets or lozenges, each containing a predetermined amount of the active ingredient as a powder or granules; or a suspension in an aqueous liquor or non-aqueous liquid such as a syrup, an elixir, an emulsion or a draught.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine, with the active compound being in a free-flowing form such as a powder or granules which is optionally mixed with a binder, disintegrant, lubricant, inert diluent, surface active agent or dispersing agent. Molded tablets comprised of a mixture of the powdered active compound with a suitable carrier may be made by molding in a suitable machine.

A syrup may be made by adding the active compound to a concentrated aqueous solution of a sugar, for example sucrose to which may also be added any accessory ingredient(s). Such accessory ingredient(s) may include flavorings, suitable preservatives, an agent to retard crystallization of the sugar, and an agent to increase the solubility of any other ingredient, such as a polyhydric alcohol, for example glycerol or sorbitol.

Formulations suitable for parenteral administration conveniently comprise a sterile aqueous preparation of the active compound, which is preferably isotonic with the blood of the recipient (e.g., physiological saline solution).

Nasal spray formulations comprise purified aqueous solutions of the active compound with preservative agents and isotonic agents. Such formulations are preferably adjusted to a pH and isotonic state compatible with the nasal mucous membranes.

Formulations for rectal administration may be presented as a suppository with a suitable carrier such as cocoa butter, or hydrogenated fats or hydrogenated fatty carboxylic acids.

Ophthalmic formulations are prepared by a similar method to the nasal spray, except that the pH and isotonic factors are preferably adjusted to match that of the eye.

Topical formulations comprise the active compound dissolved or suspended in one or more media such as mineral

oil, petroleum, polyhydroxy alcohols or other bases used for topical pharmaceutical formulations.

In addition to the aforementioned ingredients, the formulations of this invention may further include one or more accessory ingredient(s) selected from diluents, buffers, flavoring agents, binders, disintegrants, surface active agents, thickeners, lubricants, preservatives (including antioxidants) and the like.

The following Examples are provided to illustrate the present invention, and should not be construed as limiting thereof. Compounds referenced by numbers refer to the compounds illustrated in the schemes above, and compounds referenced by letters refer to the compounds named in the alphabetic list above.

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## EXAMPLE 1

Synthesis of Thymidylyl[3'-5'](3'-acetylthymidylyl)  
boranephosphonate methyl ester (Compound A)

Boronated oligonucleotides were prepared according to the method described in Scheme 2. Tetrazole (0.50 g, 7.14 mmol, 5 equiv.) was dissolved in freshly dried  $\text{CH}_3\text{CN}$  (15 ml, dried by refluxing over  $\text{CaH}_2$ , followed by distillation under Argon) under argon. To this 5'-O-DMT-thymidine-3'-(Methyl-N,N-diisopropyl)phosphoramidite (1.00 g, 1.42 mmol) dissolved in 10 ml dry  $\text{CH}_3\text{CN}$  was added by a syringe. The phosphoramidite sample vial was rinsed with another 3-4 ml of dry  $\text{CH}_3\text{CN}$  and this was also transferred by a syringe to the reaction flask. To this mixture 3'-O-acetylthymidine (0.404 g, 1.42 mmol) was added and the mixture was stirred at room temperature. After 15 minutes and complete disappearance of white solid,  $\text{Me}_2\text{S} \cdot \text{BH}_3$  (0.468 ml of 10M solution in  $\text{Me}_2\text{S}$ , 3.3 equiv.) was added. After complete disappearance of phosphite peaks in the  $^{31}\text{P}$  nmr spectrum and appearance of peak due to the product, solvent was removed from the reaction mixture at room temperature under reduced pressure. The crude product was

-21-

purified by flash chromatography on silica gel using EtOAc:Acetone (8.5:1.5). Further purification was achieved by reverse phase HPLC on a C-18 column (1 inch x 25 cm) using a gradient system (25-100% B in 38 min., flow rate 5 9.2 ml/min). The solvents used for HPLC were water (Solvent A) and acetonitrile (Solvent B). Elemental analysis was: %H calc. 5.75, found 5.94; %C calc. 46.02, found 46.01; %N calc. 9.33, found 9.02; %P calc. 5.16, found 4.97.

10

## EXAMPLE 2

Synthesis of Thymidylyl[3'-5']thymidylyl  
borane-phosphonate (Compound J)

Thymidylyl[3'-5'] (3'-acetylthymidylyl)borane-phosphonate methyl ester (40.7 mg, 0.068 mmol) was taken in 15 conc.  $\text{NH}_4\text{OH}$  (10 ml) in a sealed tube and was kept on a shaker at room temperature overnight. The tube was cooled and opened. The  $\text{NH}_3$  was allowed to escape at room temperature. The solution was cooled to  $-80^\circ\text{C}$  and lyophilized to give 42 mg of crude product. The product 20 was purified by reverse phase HPLC.

## EXAMPLE 3

Synthesis of Thymidylyl[3'-5']thymidylyl  
[3',5'] (3'-acetylthymidylyl)boranephosphonate  
dimethyl ester (Compound T)

25 To a solution of tetrazole (0.368 g, 5.25 mmol, 5 equiv.) in dry  $\text{CH}_3\text{CN}$  (15 ml) was added a solution of 5'-DMT-thymidine-5'-(methyl-N,N-diisopropyl) phosphoramidite (0.750 g, 1.063 mmol). To this mixture, Thymidylyl[3'-5'] (3'-acetylthymidylyl)boranephosphate methyl ester (0.630 g, 1.049 mmol) was added and the mixture was stirred at room 30 temperature for 40 minutes. To this  $\text{Me}_2\text{S}\cdot\text{BH}_3$  (346  $\mu\text{l}$  of 10M solution in  $\text{Me}_2\text{S}$ , 3.3 equiv.) was added and the mixture was stirred at room temperature for 4 hours. The solvent was

removed and the crude product was purified by flash chromatography on silica gel using EtOAc:Acetone (8:2). Further purification was achieved by reverse phase HPLC using a gradient of 25-52.5% B in 20 min at a flow rate of 9.2 ml/min. Solvents used were water (Solvent A) and CH<sub>3</sub>CN (Solvent B).

## EXAMPLE 4

Synthesis of (C<sup>6</sup>N-Benzoyladenyl)-[3'-5']-(3'-acetylthymidyl)boranephosphonate methyl ester

10           Tetrazole (0.430 g, 6.138 mmol) was dissolved in dry CH<sub>3</sub>CN (13 ml) under Argon. To this C<sup>6</sup>N-Benzoyl-5'-O-DMT-adenine-3' (methyl-N,N-diisopropyl)phosphoramidite (1.00 g, 1.22 mmol) dissolved in CH<sub>3</sub>CN (10 ml) was added by a syringe. The phosphoramidite vial was rinsed with 3-4 ml  
15   of CH<sub>3</sub>CN which was added to the reaction mixture. To this 3'-O-acetylthymidine (0.347 g, 1.22 mmol) was added and the mixture was stirred at room temperature for 40 minutes. Me<sub>2</sub>S·BH<sub>3</sub> (403 ml of 10M solution in Me<sub>2</sub>S, 3.3 equiv.) was added to the reaction mixture and after ten minutes a small  
20   portion was taken in CDCl<sub>3</sub> for <sup>31</sup>P mnr. NMR showed formation of desired boranephosphonate, as well as small amount of unreacted intermediate phosphite. So another 100 μl of Me<sub>2</sub>BH<sub>3</sub> was added and the mixture was stirred for thirty minutes. The solvent was removed under reduced  
25   pressure and the product was partially purified by flash chromatography on silica gel using EtOAc:Acetone (8.5:1.5).

## EXAMPLE 5

Synthesis of (C<sup>4</sup>N-benzoylcytidyl)-[3'-5']-(3'-acetylthymidyl)boranephosphonate methyl ester

30           To a solution of tetrazole (0.397 g, 5.667 mmol), 5 equiv.) in freshly dried CH<sub>3</sub>CN (15 ml) under argon, was added a solution of phosphoramidite (0.900 g, 1.132 mmol)



in dry  $\text{CH}_3\text{CN}$  (5 ml). The phosphoramidite vial was rinsed with another 5 ml of  $\text{CH}_3\text{CN}$  and the solution was transferred to the reaction flask. To this mixture was added 3'-acetylthymidine (0.322 g, 1.133 mmol) and the mixture was stirred at room temperature for 40 minutes. To this,  $\text{Me}_2\text{S}\cdot\text{BH}_3$  (374 ml of 10M solution in  $\text{Me}_2\text{S}$ , 3.3 equiv.) was added and after 4.5 hours,  $^{31}\text{P}$  nmr showed complete disappearance of intermediate phosphite and presence of desired product. The solvent was removed from the reaction mixture at room temperature under reduced pressure. The product was partially purified by flash chromatography on silica gel using EtOAc:Acetone (6:4).

#### EXAMPLE 6

##### Purification of Boronated Oligonucleotides with Bases Other Than Thymidine

Boron-containing dinucleotides where one of the bases is either A or C while the other is T have been synthesized (see Examples 4 and 5 above). In two cases, the reaction appears to be more complicated than when it was performed with just T as bases. Although the  $^{31}\text{P}$  nmr shows a broad peak in the range of 116-117 ppm with very little oxidized phosphate and phosphoramidate species, tlc analysis shows a complex mixture. Both A-T and C-T dimers have been partially purified by flash chromatography.

One of the reasons for the complex mixture observed by tlc may have been the formation of amine-borane adducts of the bases. Since the reaction between  $\text{Me}_2\text{SBH}_3$  and amines or phosphites is instantaneous and there are several places on A or C where an adduct or combination of adducts can form, the large number of products observed is not unusual. Amine boranes, however, are hydrolytically less stable than phosphite borane; therefore hydrolysis with mild acid could simplify the complex mixture. This reaction has been carried out in case of the A-T dimer and the tlc was thereby simplified.

Reaction of  $\text{Me}_2\text{SBH}_3$  with 3',5'-silylated-deoxyguanosine as a model for reaction with G as one of the bases results in base-borane adduct formation. The product, however, slowly dissociates in solution to the starting nucleoside. Therefore, reaction of G containing dinucleotide phosphite with  $\text{Me}_2\text{SBH}_3$  will probably result in adduct formation at both phosphorus and one of the base nitrogens (most likely N7). But slow dissociation of base-borane adduct in solution and stability of phosphite-borane adduct will ultimately lead to pure phosphite-borane if an equivalent amount of  $\text{Me}_2\text{SBH}_3$  is used.

## EXAMPLE 7

Antihyperlipidemic Activity

Compounds to be tested were suspended in 1% aqueous carboxymethylcellulose, homogenized and administered to male  $\text{CF}_1$  mice (25 g) intraperitoneally for 16d. On days 9 and 16, blood was obtained by tail vein bleeding, and the serum was separated by centrifugation for three minutes. The serum cholesterol levels were determined by a modification of the Liebermann-Burchard reaction in accordance with known techniques. See A. Ness, et al., Clin. Chim. Acta 10, 229 (1964). Serum triglyceride levels were determined with a commercial kit, the Fisher Hycel Triglyceride Test Kit, for a different group of animals bled on day 16. The results of these antihyperlipidemic screens, for a compound dose of 8 mg/kg, are shown in Table 1 below.

TABLE 1

Compound	Percent Inhibition		
	Serum Cholesterol		Serum Triglyceride
	Day 9	Day 16	Day 16
(A)	38	46	12
(J)	-	-	-
(T)	33	32	18

## EXAMPLE 8

Anti-Inflammatory Activity

CF<sub>1</sub> male mice (~25 g) were administered test drugs at 5-40 mg/kg in 0.05% Tween 80-H<sub>2</sub>O intraperitoneally 3 hours prior to and 30 minutes prior to the injection of 0.2 ml of 1% carrageenan in 0.9% saline into the plantar surface of the right hind foot. Saline was injected into the left hind foot, which serves as a base line. After 3 hours, both feet were excised at the tibiotarsal (ankle) joint according to standard procedures. See C. Winter et al., Proc. Soc. Expt. Biol Med. 544, 111 (1962); A. Roszkowski et al., J. Pharm. Exp. Ther. 179, 114 (1971). Control mice afforded a 78±3 mg increase in paw weight. Data on the percent inhibition of the inflammatory response for a dose of 8 mg/kg are reported in Table 2 below.

TABLE 2

<u>Compound</u>	<u>Percent Inhibition</u>
(A)	37
(T)	40

20

## EXAMPLE 9

Cytotoxic Activity

The cytotoxic activity of oligonucleoside boranophosphates of the present invention was tested on the following neoplastic cell lines:

1. L1210 lymphoid leukemia cells, R. Geran et al., Cancer Chemotherapy Reports 3, 7 (1972). (grown in RPMI + 15% FBS + antibiotics).
2. Tmolt<sup>s</sup> acute lymphoblastic T cell leukemia, S. Schreiber and N. Ikemoto, Tett. Lett 29, 3211 (1988) (grown in RPMI - 1640 + 10% FBS).
3. Colon adenocarcinoma SW480 human colorectal carcinoma. A. Leibovitz et al.,

Cancer Res. 36, 4562 (1976) (grown in L15 + 10% FBS).

4. Lung bronchogenic MB-9812, S. Aronson et al., Expt. Cell Res. 61, 1 (1970) (grown in EMEM + 10% FBS + NEAA).

5. Human Osteosarcoma TE418. H. Smith et al., Int. J. Cancer 17, 219 (1976) (grown in DMEM + 10% FBS).

6. KB epidermoid oral nesopharynx. R. Geran, supra; H. Eagle, Proc. Soc. Expt. Biol. 89, 362 (1955) (grown in EMEM + 5% calf serum).

7. Hela-S, ATCC-CCL 2.2, cervical carcinoma suspended, S. Schreiber and N. Ikemoto, supra; T. Puck et al., J. Exp. Med. 103, 273 (1956) (grown in Joklik + 5% FBS, Ham's F<sub>12</sub> + 5% FBS).

8. Breast carcinoma MDA MB157, W. Nelson-Rees et al., Int. J. Cancer 16, 74 (1975) (grown in EMEM + 10% FBS + NEAA).

9. Human glioma cell EH 118 MG transformed stain of Rous sarcoma virus, J. Lutton et al., J. Biol. Chem. 254, 11181 (1979) (grown in DMEM-H + 10% FCS).

The cytotoxic screens were conducted according to NIH protocols, see E. Huang et al., J. Pharm. Sci. 61, 108 (1972), with 10<sup>4</sup> cells, growth medium, antibiotics and drugs from 0.5 to 100 µg/ml final concentration. For the L1210, Hela-S, and Tmolt, (i.e. the suspended cells), the incubations were conducted in sterile test tubes in a final volume of 1 ml for 72 hr at 37°C in a CO<sub>2</sub> incubator. The cells on the third day were still in log growth phase. The number of cells/ml are determined using trypan blue exclusion and a hemocytometer. See, e.g., R. Geran, supra. For solid tumors 1 x 10<sup>4</sup> cells are plated with 1 ml of medium + antibiotics and the other components of growth. When the controls have converged (~95%) then the density of

the cells is estimated and the ED<sub>50</sub> values calculated.  
These data are given in Table 3 below.

TABLE 3

Cytotoxicity of Oligodeoxynucleoside Boronophosphates

Cpd.	<u>L1210</u>	<u>P388</u>	<u>Tmolt</u>	<u>Adeno Carci- noma SW480</u>	<u>KB</u>	<u>Lung Bron- cho- genic</u>	<u>Hela-S</u>	<u>Osteo sar- coma</u>	<u>Glioma</u>
A	3.21	--	2.04	3.53	3.51	4.60	3.10	7.28	4.72
J		--	3.16	0.875			1.88		1.77
T	3.45	--	3.89	1.48	0.61	6.53	2.44		

The foregoing examples are illustrative of the present invention, and are not to be taken as restrictive thereof. The invention is defined by the following claims,  
with equivalents of the claims to be included therein.

-28-

## THAT WHICH IS CLAIMED IS:

1. An oligonucleoside boranophosphate comprising a chain of natural or modified ribonucleotides or deoxyribonucleotides, or a salt thereof, containing at least one boronated internucleotide phosphodiester linkage of the formula:



wherein:

W is selected from the group consisting of =O, =S, -OR', -SR', and -OCH<sub>2</sub>CH<sub>2</sub>CN, wherein R' is C1 to C3 alkyl;

X is selected from the group consisting of -BH<sub>3</sub>, -BH<sub>2</sub>R<sub>1</sub>, -BHR<sub>1</sub>R<sub>2</sub> and -BR<sub>1</sub>R<sub>2</sub>R<sub>3</sub>;

R<sub>1</sub> is selected from the group consisting of -R<sub>4</sub>, -COOH, -COOR<sub>4</sub>, -CONHR<sub>4</sub>, -CON(R<sub>4</sub>)<sub>2</sub>, -CN'R<sub>4</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>4</sub> is C1 to C18 alkyl;

R<sub>2</sub> is selected from the group consisting of -R<sub>5</sub>, -COOH, -COOR<sub>5</sub>, -CONHR<sub>5</sub>, -CON(R<sub>5</sub>)<sub>2</sub>, -CN'R<sub>5</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>5</sub> is C1 to C18 alkyl; and

R<sub>3</sub> is selected from the group consisting of C1 to C3 alkyl.

2. A compound according to claim 1, wherein W is =O.

3. A compound according to claim 1, wherein W is -OCH<sub>3</sub>.

4. A compound according to claim 1, wherein X is selected from the group consisting of  $\text{-BH}_3$ ,  $\text{-BH}_2\text{R}_1$ , and  $\text{-BHR}_1\text{R}_2$ .

5. A compound according to claim 1, wherein X is selected from the group consisting of  $\text{-BH}_3$  and  $\text{-BH}_2\text{R}_1$ .

6. A compound according to claim 1, wherein X is  $\text{-BH}_3$ .

7. A compound according to claim 1, wherein  $\text{R}_1$  is selected from the group consisting of  $\text{-R}_4$ ,  $\text{-COOH}$ ,  $\text{-COOR}_4$ ,  $\text{-CONHR}_4$ ,  $\text{-CON(R}_4)_2$ ,  $\text{-CN}^+\text{R}_4$  and  $\text{-CN}$ , wherein  $\text{R}_4$  is C1 to C9 alkyl.

8. A compound according to claim 1, wherein  $\text{R}_1$  is selected from the group consisting of  $\text{-R}_4$ ,  $\text{-COOH}$ ,  $\text{-COOR}_4$ ,  $\text{-CONHR}_4$ ,  $\text{-CON(R}_4)_2$ , and  $\text{-CN}$ , wherein  $\text{R}_4$  is C1 to C3 alkyl.

9. A compound according to claim 1, wherein  $\text{R}_2$  is selected from the group consisting of  $\text{-R}_3$ ,  $\text{-COOH}$ ,  $\text{-COOR}_3$ ,  $\text{-CONHR}_3$ ,  $\text{-CON(R}_3)_2$ ,  $\text{-CN}^+\text{R}_3$ , and  $\text{-CN}$ , wherein  $\text{R}_3$  is C1 to C9 alkyl.

10. A compound according to claim 1, wherein  $R_2$  is selected from the group consisting of  $-R_3$ ,  $-\text{COOH}$ ,  $-\text{COOR}_3$ ,  $-\text{CONHR}_3$ ,  $-\text{CON}(\text{R}_3)_2$ , and  $-\text{CN}$ , wherein  $R_3$  is C1 to C3 alkyl.

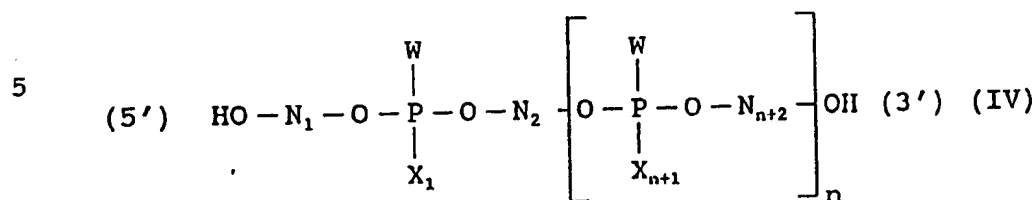
11. A compound according to claim 1, wherein  $R_3$  is methyl.

12. A compound according to claim 1, which compound comprises a chain of natural or modified deoxyribonucleotides.

13. A compound according to claim 1, which compound is capable of binding to a polynucleotide selected from the group consisting of polyribonucleic acid and polydeoxyribonucleic acid.



14. An oligonucleoside boranophosphate, or a salt thereof, having the formula, from 5' to 3', of:



10 wherein:

$n$  is an integer having a value of from 0 to 50;

$W$  is selected from the group consisting of  $=\text{O}$ ,  $=\text{S}$ ,  $-\text{OR}'$ ,  $-\text{SR}'$ , and  $-\text{OCH}_2\text{CH}_2\text{CN}$ , wherein  $R'$  is C1 to C3 alkyl;

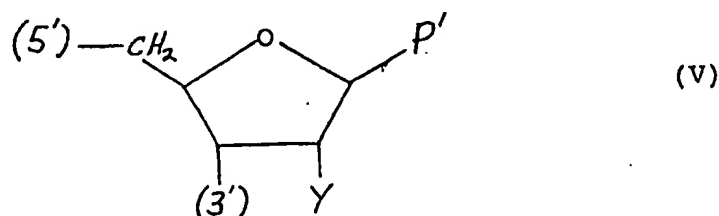
15  $X_1$  through  $X_{n+1}$  are each independently selected from the group consisting of  $-\text{OH}$ ,  $-\text{BH}_3$ ,  $-\text{BH}_2\text{R}_1$ ,  $-\text{BHR}_1\text{R}_2$  and  $-\text{BR}_1\text{R}_2\text{R}_3$ , subject to the proviso that at least one of  $X_1$  through  $X_{n+1}$  is not  $-\text{OH}$ ;

20  $R_1$  is selected from the group consisting of  $-\text{R}_4$ ,  $-\text{COOH}$ ,  $-\text{COOR}_4$ ,  $-\text{CONHR}_4$ ,  $-\text{CON}(\text{R}_4)_2$ , and  $-\text{CN}^+\text{R}_4$ ,  $-\text{CN}$ , carboxycholesteryl and carboxybenzyl, wherein  $R_4$  is C1 to C18 alkyl;

25  $R_2$  is selected from the group consisting of  $-\text{R}_5$ ,  $-\text{COOH}$ ,  $-\text{COOR}_5$ ,  $-\text{CONHR}_5$ ,  $-\text{CON}(\text{R}_5)_2$ , and  $-\text{CN}^+\text{R}_5$ ,  $-\text{CN}$ , carboxycholesteryl and carboxybenzyl, wherein  $R_5$  is C1 to C18 alkyl; and

$R_3$  is selected from the group consisting of C1 to C3 alkyl;

30  $N_1$  through  $N_{n+2}$  are each independently a nucleoside or deoxynucleoside monomer of the formula:



35

wherein  $Y$  is selected from the group consisting of  $-\text{H}$  and  $-\text{OH}$ ; and

wherein P' is a base of a natural or modified  
40 nucleic acid selected from the group consisting of adenine, thymine, cytosine, guanine, uracil, xanthine, hypoxanthine, 5-methylcytosine, 5-bromouracil, 8-azidoadenine, and 2,6-diaminopurine.

15. A compound according to claim 14, wherein P' is selected from the group consisting of adenine, thymine, cytosine, guanine, and uracil.

16. A compound according to claim 14, wherein n is from 0 to 30.

17. A compound according to claim 14, wherein n is from 0 to 18.

18. A compound according to claim 14, wherein W is =O.

19. A compound according to claim 14, wherein W is -OCH<sub>3</sub>.

20. A compound according to claim 14, wherein X<sub>1</sub> through X<sub>n+1</sub> are each independently selected from the group consisting of -OH, -BH<sub>3</sub>, -BH<sub>2</sub>R<sub>1</sub>, and -BHR<sub>1</sub>R<sub>2</sub>.

21. A compound according to claim 14, wherein X<sub>1</sub> through X<sub>n+1</sub> are each independently selected from the group consisting of -OH, -BH<sub>3</sub>, and -BH<sub>2</sub>R<sub>1</sub>.

22. A compound according to claim 14, wherein at least  $X_1$  and  $X_{n+1}$  are not -OH.

23. A compound according to claim 14, wherein  $X_1$  through  $X_{n+1}$  are not -OH.

24. A compound according to claim 14, wherein  $X_1$  through  $X_{n+1}$  is  $-BH_3$ .

25. A compound according to claim 14, wherein  $R_1$  is selected from the group consisting of  $-R_4$ ,  $-COOH$ ,  $-COOR_4$ ,  $-CONHR_4$ ,  $-CON(R_4)_2$ ,  $-CN^+R_4$ , and  $-CN$ , wherein  $R_4$  is C1 to C9 alkyl.

26. A compound according to claim 14, wherein  $R_1$  is selected from the group consisting of  $-R_4$ ,  $-COOH$ ,  $-COOR_4$ ,  $-CONHR_4$ ,  $-CON(R_4)_2$ , and  $-CN$ , wherein  $R_4$  is C1 to C3 alkyl.

27. A compound according to claim 14, wherein  $R_2$  is selected from the group consisting of  $-R_5$ ,  $-COOH$ ,  $-COOR_5$ ,  $-CONHR_5$ ,  $-CON(R_5)_2$ ,  $-CN^+R_5$ , and  $-CN$ , wherein  $R_5$  is C1 to C9 alkyl.

28. A compound according to claim 14, wherein  $R_2$  is selected from the group consisting of  $-R_3$ ,  $-\text{COOH}$ ,  $-\text{COOR}_3$ ,  $-\text{CONHR}_3$ ,  $-\text{CON}(\text{R}_3)_2$ , and  $-\text{CN}$ , wherein  $R_3$  is C1 to C3 alkyl.

29. A compound according to claim 14, wherein  $R_3$  is methyl.

30. A compound according to claim 14, wherein Y is  $-\text{H}$ .

31. A compound according to claim 14, which compound is capable of binding to a polynucleotide selected from the group consisting of polyribonucleic acid and polydeoxyribonucleic acid.

32. A method of inhibiting transcription or translation from a predetermined polydeoxyribonucleic acid or polyribonucleic acid sequence in a cell, comprising contacting an antisense agent which selectively binds to said predetermined sequence to said cell so that the antisense agent is taken into said cell, binds to said predetermined sequence, and blocks transcription or translation therefrom, wherein said antisense agent is an oligonucleoside boranophosphate comprising a chain of natural or modified ribonucleotides or deoxyribonucleotides, or a salt thereof, containing at least one boronated internucleotide phosphodiester linkage of the formula:



wherein:

W is selected from the group consisting of =O, =S, -OR', and -SR', wherein R' is C1 to C3 alkyl;

X is selected from the group consisting of -BH<sub>3</sub>, -BH<sub>2</sub>R<sub>1</sub>, -BHR<sub>1</sub>R<sub>2</sub> and -BR<sub>1</sub>R<sub>2</sub>R<sub>3</sub>;

R<sub>1</sub> is selected from the group consisting of -R<sub>4</sub>, -COOH, -COOR<sub>4</sub>, -CONHR<sub>4</sub>, -CON(R<sub>4</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>4</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>4</sub> is C1 to C18 alkyl;

R<sub>2</sub> is selected from the group consisting of -R<sub>5</sub>, -COOH, -COOR<sub>5</sub>, -CONHR<sub>5</sub>, -CON(R<sub>5</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>5</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>5</sub> is C1 to C18 alkyl; and

R<sub>3</sub> is selected from the group consisting of C1 to C3 alkyl.

33. A method of detecting a predetermined polydeoxyribonucleic acid (DNA) or polyribonucleic acid (RNA) sequence in a substrate DNA or RNA sample suspected of containing the same, comprising contacting a probe which  
 5 selectively binds to said predetermined sequence to said sample, removing unbound probe from said sample, and exposing the sample to thermal neutron radiation so that the presence of said predetermined sequence is indicated by the emission of alpha particles from said sample, the probe  
 10 comprising an oligonucleoside boranophosphate comprising a chain of natural or modified ribonucleotides or deoxyribonucleotides, or a salt thereof, containing at least one boronated internucleotide phosphodiester linkage of the formula:

15



20

wherein:

W is selected from the group consisting of =O, =S, -OR', and -SR', wherein R' is C1 to C3 alkyl;

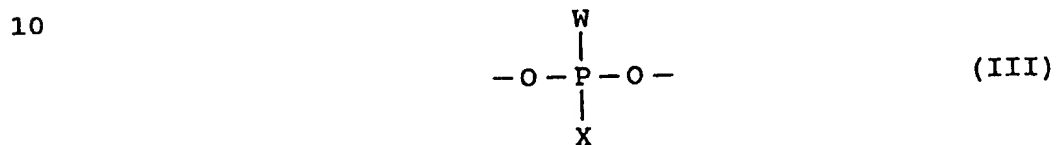
25 X is selected from the group consisting of -BH<sub>3</sub>, -BH<sub>2</sub>R<sub>1</sub>, -BHR<sub>1</sub>R<sub>2</sub> and -BR<sub>1</sub>R<sub>2</sub>R<sub>3</sub>;

R<sub>1</sub> is selected from the group consisting of -R<sub>4</sub>, -COOH, -COOR<sub>4</sub>, -CONHR<sub>4</sub>, -CON(R<sub>4</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>4</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>4</sub> is C1 to  
 30 C18 alkyl;

R<sub>2</sub> is selected from the group consisting of -R<sub>5</sub>, -COOH, -COOR<sub>5</sub>, -CONHR<sub>5</sub>, -CON(R<sub>5</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>5</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>5</sub> is C1 to C18 alkyl; and

35 R<sub>3</sub> is selected from the group consisting of C1 to C3 alkyl.

34. A method of treating inflammation in an animal subject in need of such treatment, comprising administering the subject an inflammation-combating amount of an oligonucleoside boranophosphate comprising a chain of natural or modified ribonucleotides or deoxyribonucleotides, or a pharmaceutically acceptable salt thereof, containing at least one boronated internucleotide phosphodiester linkage of the formula:



15

wherein:

W is selected from the group consisting of =O, =S, -OR', and -SR', wherein R' is C1 to C3 alkyl;

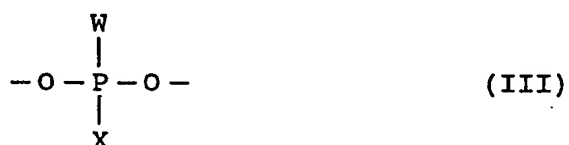
X is selected from the group consisting of -BH<sub>3</sub>, -BH<sub>2</sub>R<sub>1</sub>, -BHR<sub>1</sub>R<sub>2</sub> and -BR<sub>1</sub>R<sub>2</sub>R<sub>3</sub>;

R<sub>1</sub> is selected from the group consisting of -R<sub>4</sub>, -COOH, -COOR<sub>4</sub>, -CONHR<sub>4</sub>, -CON(R<sub>4</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>4</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>4</sub> is C1 to C18 alkyl;

R<sub>2</sub> is selected from the group consisting of -R<sub>5</sub>, -COOH, -COOR<sub>5</sub>, -CONHR<sub>5</sub>, -CON(R<sub>5</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>5</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>5</sub> is C1 to C18 alkyl; and

R<sub>3</sub> is selected from the group consisting of C1 to C3 alkyl.

35. A method of treating hyperlipidemia in an animal subject in need of such treatment, comprising administering the subject a hyperlipidemia-combatting amount of an oligonucleoside boranophosphate comprising a chain of natural or modified ribonucleotides or deoxyribonucleotides, or a pharmaceutically acceptable salt thereof, containing at least one boronated internucleotide phosphodiester linkage of the formula:



wherein:

W is selected from the group consisting of =O, =S, -OR', and -SR', wherein R' is C1 to C3 alkyl;

X is selected from the group consisting of -BH<sub>3</sub>, -BH<sub>2</sub>R<sub>1</sub>, -BHR<sub>1</sub>R<sub>2</sub> and -BR<sub>1</sub>R<sub>2</sub>R<sub>3</sub>;

R<sub>1</sub> is selected from the group consisting of -R<sub>4</sub>, -COOH, -COOR<sub>4</sub>, -CONHR<sub>4</sub>, -CON(R<sub>4</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>4</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>4</sub> is C1 to C18 alkyl;

R<sub>2</sub> is selected from the group consisting of -R<sub>5</sub>, -COOH, -COOR<sub>5</sub>, -CONHR<sub>5</sub>, -CON(R<sub>5</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>5</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>5</sub> is C1 to C18 alkyl; and

R<sub>3</sub> is selected from the group consisting of C1 to C3 alkyl.



36. A method of treating tumors in an animal subject in need of such treatment, comprising administering the subject a tumor-combating amount of an oligonucleoside boranophosphate comprising a chain of natural or modified  
 5 ribonucleotides or deoxyribonucleotides, or a pharmaceutically acceptable salt thereof, containing at least one boronated internucleotide phosphodiester linkage of the formula:



15 wherein:

W is selected from the group consisting of =O, =S, -OR', and -SR', wherein R' is C1 to C3 alkyl;

20 X is selected from the group consisting of -BH<sub>3</sub>, -BH<sub>2</sub>R<sub>1</sub>, -BHR<sub>1</sub>R<sub>2</sub> and -BR<sub>1</sub>R<sub>2</sub>R<sub>3</sub>;

R<sub>1</sub> is selected from the group consisting of -R<sub>4</sub>, -COOH, -COOR<sub>4</sub>, -CONHR<sub>4</sub>, -CON(R<sub>4</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>4</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>4</sub> is C1 to C18 alkyl;

25 R<sub>2</sub> is selected from the group consisting of -R<sub>5</sub>, -COOH, -COOR<sub>5</sub>, -CONHR<sub>5</sub>, -CON(R<sub>5</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>5</sub>, -CN, carboxycholesteryl and carboxybenzyl; wherein R<sub>5</sub> is C1 to C18 alkyl; and

30 R<sub>3</sub> is selected from the group consisting of C1 to C3 alkyl.

37. A pharmaceutical composition comprising a pharmaceutically acceptable carrier in combination with an effective amount of an oligonucleoside boranophosphate comprising a chain of natural or modified ribonucleotides or deoxyribonucleotides, or a pharmaceutically acceptable salt thereof, containing at least one boronated internucleotide phosphodiester linkage of the formula:

10



wherein:

15

W is selected from the group consisting of =O, =S, -OR', and -SR', wherein R' is C1 to C3 alkyl;

X is selected from the group consisting of -BH<sub>3</sub>, -BH<sub>2</sub>R<sub>1</sub>, -BHR<sub>1</sub>R<sub>2</sub> and -BR<sub>1</sub>R<sub>2</sub>R<sub>3</sub>;

20

R<sub>1</sub> is selected from the group consisting of -R<sub>4</sub>, -COOH, -COOR<sub>4</sub>, -CONHR<sub>4</sub>, -CON(R<sub>4</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>4</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>4</sub> is C1 to C18 alkyl;

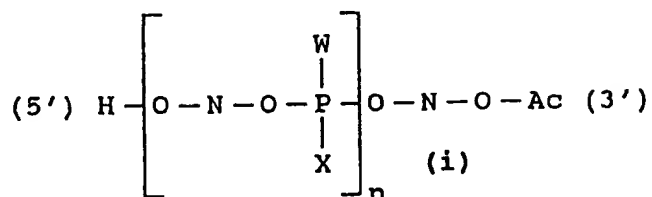
25

R<sub>2</sub> is selected from the group consisting of -R<sub>5</sub>, -COOH, -COOR<sub>5</sub>, -CONHR<sub>5</sub>, -CON(R<sub>5</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>5</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>5</sub> is C1 to C18 alkyl; and

R<sub>3</sub> is selected from the group consisting of C1 to C3 alkyl.

38. A method of making an oligonucleoside boranophosphate, comprising

(a) condensing a compound of formula (i)



wherein:

Ac is an acyl group or a solid support;

n is from 0 to 50;

N is a natural or modified ribonucleotide or deoxyribonucleotide;

W is selected from the group consisting of =O, =S, -OPr, and -SPr where Pr is a base-labile protecting group;

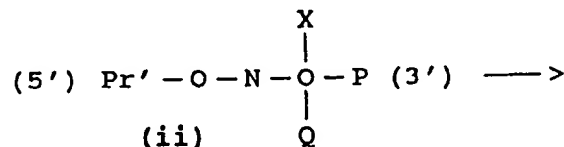
X is selected from the group consisting of -OH, -BH<sub>3</sub>, -BH<sub>2</sub>R<sub>1</sub>, -BHR<sub>1</sub>R<sub>2</sub> and -BR<sub>1</sub>R<sub>2</sub>R<sub>3</sub>;

R<sub>1</sub> is selected from the group consisting of -R<sub>4</sub>, -COOH, -COOR<sub>4</sub>, -CONHR<sub>4</sub>, -CON(R<sub>4</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>4</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>4</sub> is C1 to C18 alkyl;

R<sub>2</sub> is selected from the group consisting of -R<sub>5</sub>, -COOH, -COOR<sub>5</sub>, -CONHR<sub>5</sub>, -CON(R<sub>5</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>5</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>5</sub> is C1 to C18 alkyl; and

R<sub>3</sub> is selected from the group consisting of C1 to C3 alkyl,

with a compound of formula (ii)



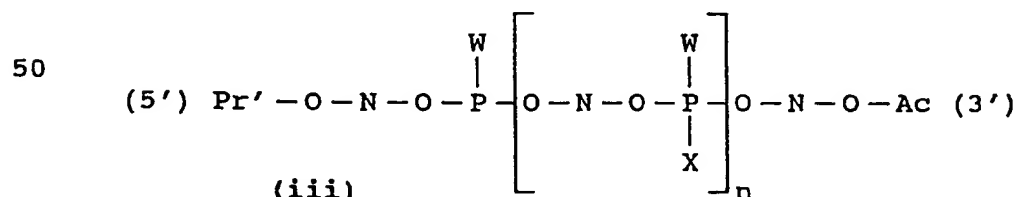
wherein:

Pr' is a protecting group;

40 N is a natural or modified ribonucleotide or deoxyribonucleotide;

W is selected from the group consisting of =O, =S, -OPr, and -SPr where Pr is a base-labile protecting group; and

45 Q is a proton acceptor;  
to produce a compound of formula (iii),



55 and then;

(b) oxidizing the compound of formula (iii) with a compound of the formula LX, wherein

L is a Lewis base; and

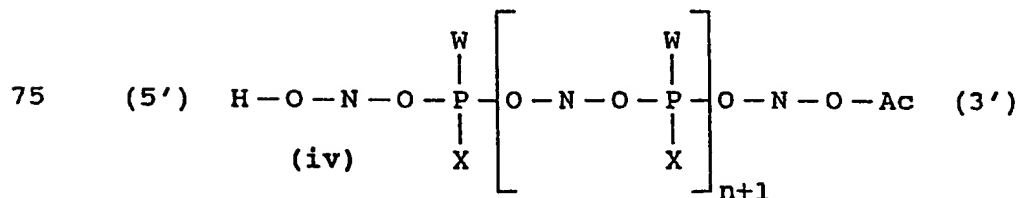
60 X is selected from the group consisting of -BH<sub>3</sub>, -BH<sub>2</sub>R<sub>1</sub>, -BHR<sub>1</sub>R<sub>2</sub> and -BR<sub>1</sub>R<sub>2</sub>R<sub>3</sub>; wherein:

R<sub>1</sub> is selected from the group consisting of -R<sub>4</sub>, -COOH, -COOR<sub>4</sub>, -CONHR<sub>4</sub>, -CON(R<sub>4</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>4</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>4</sub> is C1 to C18 alkyl;

65 R<sub>2</sub> is selected from the group consisting of -R<sub>5</sub>, -COOH, -COOR<sub>5</sub>, -CONHR<sub>5</sub>, -CON(R<sub>5</sub>)<sub>2</sub>, -CN<sup>+</sup>R<sub>5</sub>, -CN, carboxycholesteryl and carboxybenzyl, wherein R<sub>5</sub> is C1 to C18 alkyl; and

70 R<sub>3</sub> is selected from the group consisting of C1 to C3 alkyl,

to give a compound of formula (iv)



and then

- 80 (c) repeating at least step (a) by substituting the compound of formula (iv) for the compound of formula (i) in step (a).

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/06949

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>3</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC IPC(5):C07 H 1/00,21/00, 23/00; A61K 31/00, 48/00 U.S. CL.: 435/6; 514/44,64; 536/17.1, 27, 28, 29		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>4</sup>		
Classification System	Classification Symbols	
U.S.	514/44, 64; 536/17.1, 27, 28, 29; 435/6	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>5</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> <sup>14</sup>		
Category <sup>6</sup>	Citation of Document, <sup>16</sup> with indication, where appropriate, of the relevant passages <sup>17</sup>	Relevant to Claim No. <sup>18</sup>
A	US, A, 3,687,808 (Merigan, Jr., et al) 14 August 1969, see entire document.	1-38
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>15</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search <sup>1</sup>		Date of Mailing of this International Search Report <sup>2</sup>
05 March 1991		<b>23 APR 1991</b>
International Searching Authority <sup>1</sup>		Signature of Authorized Officer <sup>10</sup>
ISA/US		<i>James O. Wilson for</i> James O. Wilson <span style="float: right;">ebw</span>